ABSTRACT

Ph.D Thesis

PROPAGATION OF *Rhaponticoides mykalea* (Hub.-Mor.) M. V. Agab. & Greuter IN *IN VITRO* CONDITIONS AND INVESTIGATION OF THE FACTORS AFFECTING THESE PROCESSES

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In this study, the factors have been determined which threatened *Rhaponticoides mykalea* (Hub.-Mor.) species in the under critical danger (CR); processes to be exceeded during propagation with *in vitro* tissue culture techniques and changes in antioxidan enzymes which effect these processes have been investigated.

First, the factors which have effect on germination of seeds have been researched. In the experiments of *in vitro* seed germination, seeds after 8 month keeping period were cultured on distilled water medium adjusted to 7.5 pH and have been cultured in 18 °C. In this experiments maximum germination percentage (30 %) has been obtained.

Mature and immature zygotic embryos have been used as explant for embryo culture experiments. As a result, seedlings have been obtained from mature zygotic embryos on the 0.01 mg/L Thidiazuron (TDZ) added Murashige and Skoog (MS) medium (63.33 %).

Immature zygotic embryos have been used as explant for somatic embryogenesis experiments. The highest callus formation rate has been obtained in 0.25 mg/L α-naphthaleneacetic acid (NAA) and 1 mg/L ^{6}N-benzyl adenine (BA) added MS medium (75 %). The highest embryo differantiation has been obtained in 0.1 mg/L BA and 6 % sucrose added MS medium (19.25 somatic embryo/callus). Somatic embryos differantiated and devoloped in MS medium with high sucrose (12 %) have been converted to plantlets (15.75 %).

The shoots were obtained from embryo culture experiments have been used for axillary shoot propagation experiments. The highest axillary shoot number has been obtained in 0.5 mg/L BA added MS medium (5.8 shoot/explant). 0.1 mg/L Kinetin

(KIN) added MS medium was determined as the the most suitable medium for the maximum shoot length (7.35 cm).

The leaves which were collected from nature were used as explant for adventitious shoot propagation experiments. The highest callusing percentage has been obtained in the 1.0 mg/L NAA added MS medium (75 %). The highest shoot formation from calli has been obtained in 0.5 mg/L NAA and 2 mg/L BA added medium (4.2 shoot/callus). The longest shoots were formed in 0.1 mg/L NAA and 4 mg/L BA added medium (6.3 cm).

The shoots obtained at the end of axillary and adventitious shoot multiplication were rooted and transffered to external environment step by step. The optimum rooting concentration was ½ MS+ 0.5mg/L Indole-3-butyric acid (IBA) in each experiment.

Besides, antioxidan enzyme activities such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) have been determined during the organogenesis and somatic embryogenesis processes. During the callus formation from leaf explants in organogenesis process, SOD activity have been increased gradually, and a graded decrease has been observed during the shoot regeneration from callus. First week of culture, PO activity is higher than CAT activity. Both enzyme activities have been decreased during the callus formation process. CAT and PO activities have been increased dependent on the adventitious shoot formation.

The activities of SOD increased gradually during the embryogenic callus formation which is the early phase of somatic embryogenesis. The activities of SOD began to decline step by step when the globular structures occured on calli. It has been observed that SOD activity has been decreased because of the embryo differentiation increase. First week of culture, CAT activity was higher than PO activity. A graded decrease of both enzyme activities has been observed during the the embryogenic callus formation. It has been observed that both PO and CAT avtivity have been increased during the globular structure formation which is the phase of embryogenesis process and somatic embryo differentiation.

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Key Words

R.mykalea, CR endemic plant, *in vitro*, axillary shoot, adventitious shoot, embryo culture, somatic embryogenesis, antioxidan enzymes